CLAIMS

1. A method for the screening of compounds that modulate calcium release-activated channel (Icrac) activity, comprising:

   a. contacting a test compound and a selective calcium channel activator, with a population of calcium channel expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter, and

   b. determining the activity of the test compound on a calcium release-activated channel by measuring the reporter gene expression in said cells.

2. The method of claim 1, wherein, in step a), the selective calcium channel activator is an Icrac activator and the calcium channel expressing cells are Icrac expressing cells.

3. The method of claim 2, wherein, in step a), the cells are contacted with an Icrac activator in the absence of a Protein Kinase C activator.

4. The method of claim 2, wherein the Icrac activator is a product or a treatment that selectively depletes intracellular calcium stores.

5. The method of claim 4, wherein the Icrac activator is thapsigargin.

6. The method of claim 1, wherein the reporter gene is a β-lactamase gene.

7. The method of claim 1, wherein the NFAT-inducible promoter is a transcriptional promoter comprising a NFAT-responsive region.

8. The method of claim 7, wherein the NFAT-inducible promoter comprises one or several copies of the nucleotide sequence of SEQ ID N° 1.

9. The method of claim 8, wherein the NFAT-inducible promoter comprises between 2 and 8 copies of the nucleotide sequence of SEQ ID N° 1.

10. A method for the screening compounds that modulate calcium release-activated channel (Icrac) activity comprising :

(a) contacting a test compound and a selective, direct or indirect, Icrac activator with a population of Icrac expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter,

(b) contacting the cells of a) with a substrate of the reporter gene, and

(c) determining the activity of the test compound on the calcium release-activated channel by assessing the hydrolysis of the substrate in said cells.

11. The method of claim 10, wherein the reporter gene is a β-lactamase gene under the control of a NFAT-inducible promoter and the substrate is the substrate of β-lactamase,

12. The method of claim 10, wherein, in step b), the substrate is a ratiometric substrate.

13. The method of claim 12, wherein the substrate is CCF2-AM.

14. The method of claim 1, wherein the population of cells comprises a culture of blood cells selected from lymphocytes, mastocytes, or dendritic cells.

15. The method of claim 1, wherein the population of cells comprises between 10^3 and 10^6 cells.

16. The method of claim 1, wherein the test compound and the Icrac activator are contacted simultaneously with the cells.

17. The method of claim 1, wherein at least two test compounds are contacted in parallel with the cell population.

18. The method of claim 17 wherein at least 10 compounds are contacted in parallel.

19. The method of claim 17 wherein at least 50 compounds are contacted in parallel.

20. The method of claim 1, wherein step a) is performed in a multi-well plate.

21. The method of claim 1, wherein the contact time between the test compound and the Icrac activator with the cells is from 2 to 6 hours.

22. The method of claim 1, wherein the cell population is incubated in a medium having a calcium concentration of at least 1 mM .

23. The method of claim 1, wherein said method is used for assaying the activity of a test compound.

24. A method for the screening of Icrac blockers, comprising:

a. contacting a test compound and an Icrac activator with a population of Icrac-expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter, said cells being incubated in a medium having a calcium concentration of at least 1 mM ,

b. contacting the cells of a) with a substrate of the reporter gene expression product, and

c. determining the activity of the test compound on the calcium release-activated channel by assessing the hydrolysis of the substrate in said cells.

25. The method of claim 24, wherein the reporter gene is the β-lactamase gene.

26. The method of claim 24, wherein the cells are incubated in a medium lacking phorbol ester.

27. A method for the screening of Icrac stimulators, comprising:

a) contacting a test compound with a population of Icrac-expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter, said cells being incubated in a medium having a calcium concentration of at least 1 mM ,

b) contacting the cells of a) with a substrate of the reporter gene expression product, and

c) determining the activity of the test compound on the calcium release-activated channel by assessing the hydrolysis of the substrate in said cells.

28. The method of claim 27, wherein the reporter gene is β-lactamase gene.

29. The method of claim 27, wherein the cells are incubated in a medium lacking phorbol ester.

30. The method of claim 1, for screening a compound that modulates the activation of Icrac.

31. The method of claim 1, for screening a compound that modulates the Icrac-mediated calcium inflow.

32. A method for the screening of compounds that inhibit calcium release-activated channel (Icrac) activity comprising :

(a) contacting at least a test compound and a selective, direct or indirect, Icrac activator with a population of Icrac expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter,

(b) contacting the cells of a) with a substrate of the reporter gene,

(c) determining the activity of the test compounds on the calcium release-activated channel by assessing the hydrolysis of the substrate in said cells,

(d) selecting compounds which inhibit at least 40 % of the activity

(e) screening of the compounds obtained in d) in order to eliminate those which modulate β-lactamase activity in a non-NFAT dependent manner by contacting the compounds selected in d) with a population of cells comprising a reporter construct comprising a β-lactamase gene under the control of a non-NFAT-inducible promoter, and selecting compounds which modulate β-lactamase activity in a NFAT dependent manner.

33. The method of claim 32, wherein the reporter construct comprising a β-lactamase gene is under the control of a CRE-inducible promoter.

34. The method of claim 33, wherein the CRE-inducible promoter comprises between 1 and 8 CRE sequences.

35. A kit for use in a method according to claim 1, comprising a cell population as defined in claim 1, a support, and a substrate.

36. A blood cell or a blood-derived cell for use in a method according to claim 1, wherein said cell contains a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

37. A blood cell or a blood-derived cell for use in a method according to claim 32, wherein said cell contains a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

38. A lymphocyte or a lymphocyte-derived cell for use in a method according to claim 1, wherein said cell contains a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

39. A lymphocyte or a lymphocyte-derived cell for use in a method according to claim 32, wherein said cell contains a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

40. A mastocyte or a mastocyte-derived cell for use in a method according to claim 1, wherein said cell contains a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

41. A population of rodent immune cells for use in a method according to claim 1, wherein said cell comprises a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

42. The population of rodent immune cells of claim 41, wherein said population is a population of murine or rat immune cells.

43. The cell population of claim 41, wherein said population comprises at least 80 % of cells expressing the Icrac channel.

44. A population of human immune cells for use in a method according to claim 1, wherein said population comprises a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter.

45. The cell population of claim 44, wherein said population comprises at least 80 % of cells expressing the Icrac channel

46. The method of claim 32, wherein the non-NFAT inducible promoter is selected from CRE-inducible promoter, VIP responsive promoter, promoters containing NFκB or JNK reponsive element.

47. A method for the screening of a compound that activates calcium release-activated channel (Icrac) activity comprising :

(a) contacting at least one test compound with a population of Icrac expressing cells, said cells further containing a reporter construct comprising a reporter gene under the control of a NFAT-inducible promoter,

(b) contacting the cells of a) with a substrate of the reporter gene,

(c) determining the activity of the test compounds on the calcium release-activated channel by assessing the hydrolysis of the substrate in said cells,

(d) selecting compounds which increase at least 20 % of the activity

(e) screening of the compounds obtained in d) in order to eliminate those which modulate β-lactamase activity in a non-NFAT dependent manner by contacting the compounds selected in d) with a population of cells comprising a reporter construct comprising a β-lactamase gene under the control of a non-NFAT-inducible promoter, and selecting compounds which modulate β-lactamase activity in a NFAT dependent manner.